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Treatability Studies of Pesticide Manufacturing Wastewaters: Glyphosate

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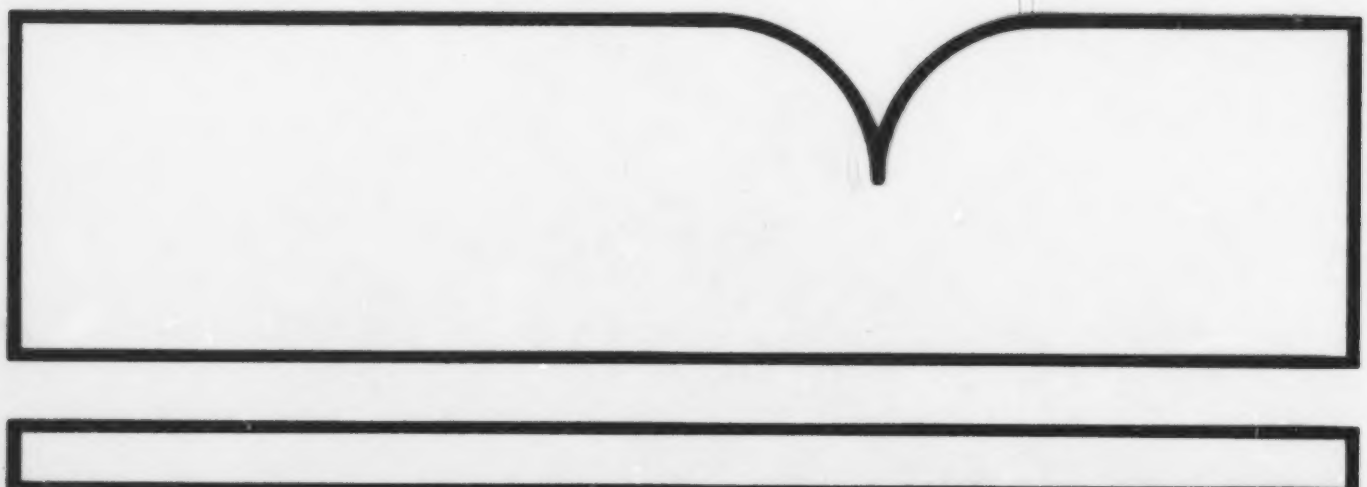

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16. ABSTRACT The report gives results of subjecting various combinations of glyphosate production wastestreams to biological treatment following lime-pretreatment to reduce high levels of glyphosate. Bench-scale biological treatment demonstrated that glyphosate did not appear to interfere with biological degradation at concentrations up to 105 mg/L. Glyphosate itself showed only partial reduction with biological reduction (28-45%). The mechanism of this removal is not fully understood but may include sorption on sludge. No evidence for metabolism of glyphosate was generated in oxygen uptake studies. While the test does not provide any evidence for metabolic uptake of glyphosate, it is interesting to note that fairly high concentrations of the compound do not inhibit other microbial processes in acclimated sludge. Biological treatment significantly reduced the toxicity of these effluents to algae (Selenastrum capricarnutum) and invertebrates (Daphnia magna). Additional treatment options were investigated in an attempt to reduce glyphosate concentrations in the biologically treated effluents. These options included ozonation, adsorption, and ion exchange. These options only marginally reduced glyphosate concentrations in biologically treated effluents.

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Pesticides	Stationary Sources	06F 06T
Industrial Processes	Glyphosate	13H
Waste Water	Treatability	06P
Waste Treatment	Chemical Oxygen Demand	07B
Ammonia	Phosphates	
Nitrogen		
Toxicity		
Oxygen Demand		

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ABSTRACT

Various combinations of glyphosate production wastestreams were subjected to biological treatment following lime-pretreatment to reduce high levels of glyphosate. Bench scale biological treatment demonstrated that glyphosate did not appear to interfere with the biological degradation process at concentrations up to 105 mg/L. On the other hand, glyphosate itself showed only partial reduction with biological treatment (28 to 45 percent). The mechanism of this removal is not fully understood but may include sorption on sludge. No evidence for metabolism of glyphosate was generated in oxygen uptake studies. While the test does not provide any evidence for metabolic uptake of glyphosate, it is also interesting to note that fairly high concentrations of the compound do not inhibit other microbial processes in acclimated sludge.

Biological treatment significantly reduced the toxicity of these effluents to algae (Selenastrum capricarnutum) and invertebrates (Daphnia magna).

Additional treatment options were investigated in an attempt to reduce glyphosate concentrations in the biologically treated effluents. These options included ozonation, adsorption, and ion exhange. These treatment options provided only marginal reduction of glyphosate concentrations in biologically treated effluents.

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SECTION 1

INTRODUCTION

In February 1979 the Research Triangle Institute (RTI) was requested by the Industrial Environmental Research Laboratory, Research Triangle Park (IERL-RTP) of the U.S. Environmental Protection Agency (EPA) to conduct laboratory and pilot studies of the treatability of pesticides manufacturing wastewaters. The project is designed to investigate the suitability of individual pesticide manufacturing wastewaters for discharge to biological treatment systems, whether publically owned treatment works (POTW) or on-site systems.

The approach taken with each pesticide manufacturing wastewater is hierarchical in nature, that is, less costly, more available methods of treatment are investigated first. The preferred method of treatment is assumed to be biological treatment. If the pesticide is judged suitable to biological treatment based on chemical and toxicological evaluation of the waste before and after treatment, additional options are not investigated.

If pesticide manufacturing wastewater disrupts biological treatment systems, the possibility of pretreating the waste prior to biological treatment is investigated. Pretreatment options may include pH adjustments, filtration, flocculation, oxidation and others depending on the nature of the waste and its chemical composition.

If pretreatment does not improve the performance of activated sludge systems, adsorption techniques may be investigated. These may involve both carbon and resin systems. Physical-chemical treatability of wastewaters will again be evaluated as with the biological treatment system.

This report details a study of the treatability of wastestreams resulting from the manufacture of glyphosate by Monsanto Agricultural Products Co., at its Luling, Louisiana facility.

SECTION 2

CONCLUSIONS

Various combinations of glyphosate production wastestreams were subjected to biological treatment following lime-pretreatment to reduce high levels of glyphosate. Bench scale biological treatment demonstrated that glyphosate did not appear to interfere with the biological degradation process at concentrations up to 105 mg/L. On the other hand, glyphosate itself showed only partial reduction with biological treatment (28 to 45 percent). The mechanism of this removal is not fully understood but may include sorption to sludge. No evidence for metabolism of glyphosate was generated in oxygen uptake studies. While the test does not provide any evidence for metabolic uptake of glyphosate, it is also interesting to note that fairly high concentrations of the compound do not inhibit other microbial processes in acclimated sludge.

Biological treatment significantly reduced the toxicity of these effluents to algae (Selenastrum capricarnutum) and invertebrates (Daphnia magna). The test data clearly show that the higher toxicity in influents versus effluents is not due to glyphosate itself but other wastestream components, many of which are effectively treated. The toxicity of glyphosate was found to be dependent on water quality parameters such as calcium and magnesium concentration; toxicity decreased as water hardness increased. Glyphosate was found to be more toxic in soft water than similar concentrations of glyphosate in the effluents from biological treatment systems. The decreased toxicity in effluents is probably related to the addition of calcium in the lime pretreatment step.

Additional treatment options were investigated in an attempt to reduce glyphosate concentrations in the biologically treated effluents. These options included ozonation, adsorption, and ion exhange. These treatment options provided only marginal reduction of glyphosate concentrations in biologically treated effluents.

SECTION 3

SELECTED LITERATURE REVIEW

GENERAL CHEMICAL INFORMATION

Chemical Name

N-(phosphonomethyl) glycine

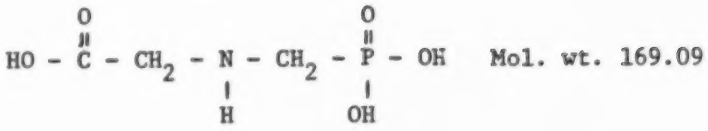
CAS Number

1071-83-6

Synonyms and Trade Names

Roundup®. MON0573

Structure



Mol. wt. 169.09

Chemical Properties

Solubility in water is about 1.9 percent by weight. The pKa's for the phosphonic acid moiety are <2.0 and 5.6. The pKa for the carboxylic group is 2.6 and for the amine is 10.6 (Sprankle et al. 1975).

Introduction and Uses

Introduced in 1971 by Monsanto as a postemergent herbicide (Baird et al., 1971). Registered with the Environmental Protection Agency for control of annual and perennial weeds before the emergence of agronomic plants (Folmar et al., 1971). Also effective in controlling ditch bank vegetation (Comes et al. 1976).

Production

The methods and reactions for production of glyphosate are proprietary. Production basically involves synthesis and isolation of an intermediate. The intermediate is then reacted to give the final glyphosate product.

Figure 1 presents a waste generation schematic from the production of glyphosate.

Aquatic Toxicity

Folmar, Sanders, and Julin (1979) conducted extensive testing of technical grade glyphosate, the isopropylamine salt of glyphosate, the formulated herbicide Roundup®, and the Roundup® Herbicide surfactant on four aquatic invertebrates and four fish species. All tests were conducted in soft water (40 mg/L as $CaCO_3$). Table 1 summarizes some of their findings. Generally it was found that technical glyphosate was considerably less toxic than Roundup® or the surfactant. The increased toxicity of Roundup® Herbicide is likely due to the presence of the surfactant in this mixture. Interspecies variation in response to an individual compound or mixture was relatively small.

Mammalian Toxicity

The acute, oral LD_{50} of the mono (dimethylamine) salt of glyphosate was found to be 9,800 mg/kg on mixed sex rats (Baird et al. 1971). The acute skin absorption minimum lethal dose of this compound for mixed-sex rabbits was greater than 7,940 mg/kg (Baird et al. 1971).

The acute oral LD_{50} for rats of glyphosate as listed in the Registry of Toxic Effects of Chemical Substances (NIOSH), 1977) is 4,320 mg/kg.

Bababunmi, Olorunsogo, and Bassir (1978) studied the toxicology of glyphosate in rats and mice. They found acute oral LD_{50} values of 4,873 mg/kg and 1,568 mg/kg in rats and mice, respectively. The intraperitoneal levels were 238 mg/kg for rats and 134 mg/kg for mice.

Fate in Soil

Glyphosate was found to be readily degraded in the soil (Sprankle, Meggitt, and Penner, 1975). These investigators found that 17 to 45 percent of ^{14}C-glyphosate was evolved as $^{14}CO_2$ in 28 days from various soil types. Sodium azide treatment of the soil reduced the rate of degradation of glyphosate indicating that at least part of the degradation was microbial. Sprankle et al., (1975) also found that glyphosate was readily bound to clay and organic matter with a corresponding limited mobility.

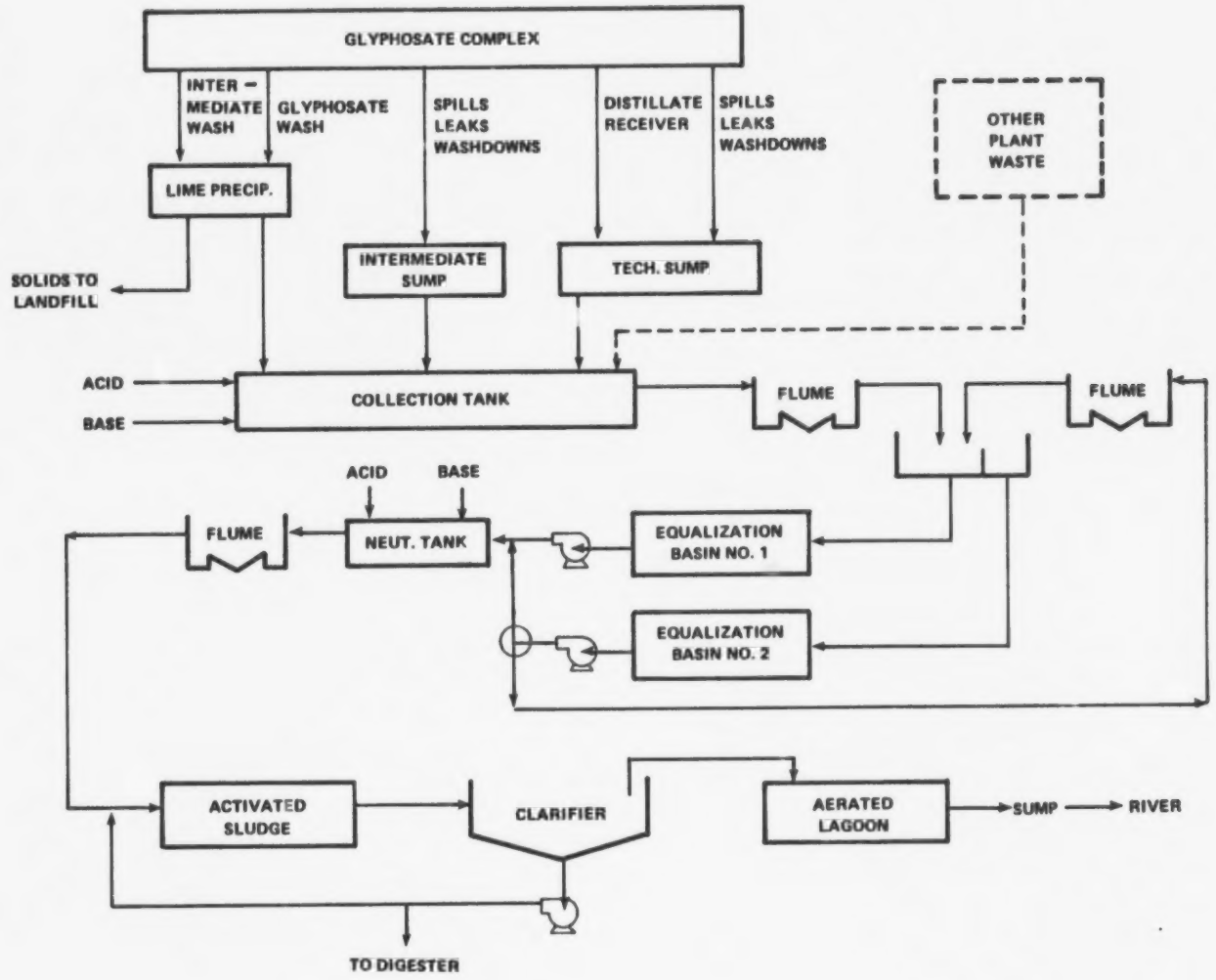


Figure 1. Wastewater production and treatment schematic for glyphosate manufacturing facility.

TABLE 1. AQUATIC TOXICITY DATA FOR GLYPOSATE AND ASSOCIATED COMPOUNDS

Organism	Temperature (°C)	Roundup® Herbicide LC50 or EC50 (mg/L)			Glyphosate LC50 or EC50 (mg/L)			Surfactant LC50 or EC50 (mg/L)		
		24h	48h	96h	24h	48h	96h	24h	48h	96h
Daphnia magna (daphnia)	22		3.0							
Gammarus pseudolimnaeus (scuds)	12		62	43						
Chironomous plumosus (midge larvae)	22		18			55			13	
Salmo gairdneri (rainbow trout)	12	8.3		8.3	140		140	2.1		2.0
Pimephales promelas (fathead minnows)	22	2.4		2.3	97		97	1.4		1.0
Ictalurus punctatus (channel catfish)	22	13		13	130		130	18		13
Lepomis macrochleus (bluegills)	22	6.4		5.0	150		140	3.0		3.0

Rueppel, Brightwell, Schaefer, and Marvel (1977) investigated the degradation of ^{14}C-labeled glyphosate and aminomethylphosphonic acid, its major soil metabolite, in soil samples. Anaerobic, aerobic and sterile conditions were studied. Under sterile conditions less than 1 percent of the ^{14}C was released in seven days indicating that chemical degradation is not a significant mode of degradation. Under anaerobic and aerobic conditions from 30 to 50 percent of the total glyphosate ^{14}C was evolved as $^{14}CO_2$ in 28 days depending on soil type. Depending on soil type the remaining activity could be accounted for primarily as the major metabolite or as soil bound nonextractable residues. Experiments with ^{14}C-labeled aminomethylphosphonic acid have demonstrated that 34.8 and 16.1 percent of the metabolite ^{14}C was evolved as $^{14}CO_2$ in 63 days with two soil types. Slower degradation of aminomethylphosphonic acid than glyphosate may reflect tighter binding to soil and/or lower cell permeability of aminomethylphosphonic acid.

TABLE 2. PESTICIDE MANUFACTURING WASTEWATER CHARACTERIZATIONS

Parameter	Intermediate wash	Glyphosate wash	Glyphosate distillate receiver
pH	<1.00	2.45	2.8
Cl^-, mg/L	82,430	4,370	5.5
Acidity, mg/L as $CaCO_3$	190,000	56,250	3,402
Total Kjeldahl nitrogen, mg/L as N	5,690	8,030	4.0
Ammonia, mg/L as N	<0.5	213	2.0
Oxidized nitrogen mg/L as N	0.6	8.4	0.7
Total phosphorous, mg/L	18,550	14,325	2.0
Orthophosphate, mg/L	1,810	1,060	0.4
Chemical oxygen demand mg/L	77,000	49,250	6,500
Total dissolved solids, mg/L	(15,100)*	---	---
Total solids, mg/L	---	(81,000)	---
Formaldehyde mg/L	present	present	present
Glyphosate mg/L	---	20,300	16**
		(20,400)	(200)**
Glyphosate mg/L intermediate	(27,000)	---	---
Total organic carbon mg/L	(44,000)	(27,000)	(3,100)

*Numbers in parenthesis were furnished by Monsanto.

**Discrepancy discussed on page 10.

and carbon. The COD test typically underestimates the TOC data due to the less efficient oxidation of some compounds by the COD procedure.

It should be expected from the data provided in Table 2 that all three wastestreams would provide a substantial challenge for typical treatment systems. The presence of formaldehyde, glyphosate, glyphosate intermediate and acidity are potential problems for the biological treatment of these wastes.

BIOLOGICAL TREATMENT WITH LIME PRETREATMENT AND pH ADJUSTMENT

Three combinations of glyphosate production wastestreams were tested for biological treatability. The three conditions were designed to test the treatability of glyphosate and associated compounds in increasingly complex matrices. These three conditions are discussed below and a summary is provided in Appendix F for easy references.

Condition I tested the biodegradibility of glyphosate in a relatively simple matrix. Distillate receiver waste was diluted 1 part to 19 parts deionized water. Based on analyses provided by Monsanto of split samples the influent would contain glyphosate at 10 mg/L and TOC (COD) at 155(325) mg/L. Subsequent glyphosate analyses of undiluted distillate receiver waste conducted at RTI gave a lower concentration than initially indicated (16 mg/L as opposed to 200 mg/L). These results were confirmed by Monsanto. The initial results apparently reflect an unknown positive interference which affects one of the two methods used by Monsanto for the analysis of glyphosate. To compensate for the reduced levels of glyphosate it was decided on day 35 of biological treatment to begin spiking Condition I influent with a solution of technical grade glyphosate (12,300 mg/L as glyphosate) to give a final glyphosate concentration of 25 mg/L.

Condition II tested a combination of distillate receiver and glyphosate wash. To reduce the concentration of glyphosate in the glyphosate wash an initial lime precipitation treatment was employed. This process is similar to a system used by Monsanto as noted in Figure 1. Calcium hydroxide was added to centrifuge wash at a rate of 40 grams per 100 mls solution which produces a slurry since only a small fraction of the lime actually dissolves. The addition of this quantity of lime produces a

considerable heat of solution. To duplicate the higher temperatures of a full scale system, which may improve removal rates, laboratory treatment was conducted in a water bath at 80°C. Contact time was approximately 5 min before vacuum filtration through Whatman 1 filter paper. Glyphosate levels in the glyphosate wash were reduced from 20,300 mg/L to 2,100-2,600 mg/L. Phosphate levels also showed a large reduction. Orthophosphate levels were reduced from 1,060 mg/L to 54 mg/L as P and total phosphorous levels were reduced from 14,325 to 225 mg/L as P. COD of the glyphosate wash was reduced from 49,250 to 15,240 mg/L with lime treatment.

The lime precipitation step will produce a substantial quantity of solid waste. Currently, Monsanto is treating with lime at a lower rate with no apparent loss in removal effectiveness. While the quantity of solid waste would be decreased the actual concentration of glyphosate in the solid waste would be increased.

To obtain Condition II influent, 37.5 mls of lime-treatment glyphosate wash was added to 962.5 mls of Condition I influent. Influent glyphosate concentration ranged from 100 to 105 mg/L. COD levels of the influent averaged 770 mg/L.

Condition III influent tested the treatability of a combination of distillate receiver waste, lime-treated glyphosate wash, and intermediate wash. The purpose of Condition III was to test any effect of glyphosate intermediate and associated compounds on the biodegradability of glyphosate. It should be noted that effluent guidelines for the treatment of wastewaters from the production of pesticide intermediates were not issued by EPA under the so-called "best practical treatment" (BPT) standards. The status of pesticide intermediate wastewaters under future regulations has not been decided. In anticipation of any change in status it is important to obtain information on the characteristics of these pesticide intermediates.

The synthesis of glyphosate can be accomplished by several different methods. The actual method for the commercial synthesis of glyphosate is proprietary. For this reason the manufacturer was reluctant to reveal the identity of the intermediate. In addition, a routine method for the

analysis of the glyphosate intermediate is not available and could not be developed within the constraints of this study. Since it would not be possible to monitor the effectiveness of the lime pretreatment of the intermediate wash, it was decided to avoid the uncertainty in the intermediate concentration by diluting the intermediate wash with distilled water to approximate the concentration of intermediate reported by the manufacturer in the influent to biological treatment. The glyphosate intermediate concentrations in Condition III were therefore obtained by calculation based on analyses by the manufacturer rather than independent analyses.

Given the lack of suitable analytical methods for low concentrations of glyphosate intermediate, analyses of biologically treated Condition III effluent were not possible. Various indirect measures could be taken including the effect of the intermediate on biodegradation of glyphosate, effect on COD reduction, and effect on toxicity relative to Conditions I and II.

To obtain Condition III influent 18 mL of intermediate wash were added to 982 mL of Condition II influent. This dilution was calculated to give a glyphosate intermediate concentration of 500 mg/L. After additional discussion with Monsanto personnel and review of effluent data for the Luling facility, it was decided that 250 mg/L of intermediate was closer to a typical value which might be expected in the effluent from glyphosate manufacture. This concentration would allow for a significant input of intermediate from the "spills, leaks, and washdowns." From day 5 of biological treatment the input of intermediate wash to Condition III influent was cut to 9 mL/L. This change reduced the COD of Condition III influent from 2,150 mg/L to about 1,500 mg/L.

Again it should be noted that Appendix F provides a summary of these three conditions.

Formaldehyde concentrations of Conditions I, II, and III were lower than would be seen in any undiluted combination of distillate receiver waste, glyphosate wash, and intermediate wash. This lower concentration is meant to reflect the loss of formaldehyde during the approximate four day residence time in the equalization basins prior to the inlet of the

SECTION 4

TREATABILITY STUDIES

CHARACTERIZATION OF WASTEWATER FROM GLYPHOSATE MANUFACTURE

The manufacture of glyphosate results in five wastestreams as is depicted in Figure 1. Two of these five wastestreams are intermittent in nature and were not sampled for this study. The two streams which were not sampled were the spills, leaks, and washdowns from the glyphosate-intermediate production unit and spills, leaks, and washdowns from the glyphosate production unit. The three streams sampled for use in the treatability study include: intermediate washwater from the production of glyphosate-intermediate; washwater from the recovery of glyphosate product; and a distillate byproduct from the final glyphosate product isolation step.

The sampling of these three streams included a three day composite of glyphosate wash; a two-day composite of glyphosate distillate receiver; and a one day composite of glyphosate intermediate wash. Over the sampling period flow rates from the different wastestreams were fairly constant and average as follows: intermediate wash, 3.0 gpm; glyphosate wash, 4.5 gpm; distillate receiver, 112 gpm.

Table 2 presents data on various parameters of these wastestreams. The numbers in parenthesis represent data supplied by Monsanto on split samples. The methods of analysis used at RTI are contained in Appendices A and B. The only large discrepancy in the two sets of numbers was the measured glyphosate concentration in the distillate receiver. This discrepancy was resolved in favor of the smaller number. A smaller discrepancy exists between the total organic carbon (TOC) data and the chemical oxygen demand (COD) data. Theoretically the COD should be 32/12 times the TOC which reflects the relative weight of oxygen (O_2)

biological aeration basin at Monsanto's Luling facility. The formaldehyde concentration is lower than expected from a simple dilution of glyphosate effluents which compose about half of the input to Monsanto's aeration basin. The actual mechanism of removal has not been confirmed and could include biological degradation and/or volatilzation. A similar occurrence was noted during the bench scale treatability studies. Influent to biological units was mixed daily. At the end of the 18 to 24 hour "shelf life" of the influent, formaldehyde levels typically dropped 50 to 75%. No formaldehyde was detected during the entire course of the study in any effluent from the three treatment conditions (detection limit of 1 mg/L).

Biological treatability of the three combinations of glyphosate wastewater was tested in bench scale units designed by Swisher (1974, see Appendix C for details of construction). Two units were run per condition. The units were innoculated with an activated sludge seed from Monsanto's biological treatment system at Luling, Louisiana. The seed was aerated, chilled in a glass container, enclosed in styrofoam and shipped to RTI by airline. Total transit time was approximately eight hours.

The initial mixed liquor volatile suspended solids levels (MLVSS) in the units ranged from 3,700 to 4,400 mg/L. The mixed liquor suspended solids (MLSS) of the units initially ranged from 10,770 to 11,830 mg/L. The MLVSS-MLSS ratio averaged about 0.38 which is about half the value of typical municipal waste treatment plant. This ratio will be discussed more completely later in the report.

Sludge age of between 15 and 20 days was maintained in Condition II and III units. Very little sludge was wasted from Condition I due to the long retention time and correspondence low food to microorganism ratio. Retention time in all units was initially set at 24 hours. After 34 days Condition II and III units were extended to 48 hour retention time. The pH of all the influents was adjusted and buffered between 7.0 and 7.4 by the addition of 0.4 grams $NaHCO_3$ per liter of influent and either 10% HCl or 10% NAOH as required. Dissolved oxygen was maintained at > 2 mg/L in the aerator section of the treatment units.

Figures 2, 3, and 4 plot influent and effluent COD concentrations of Conditions I, II, and III, respectively. The Condition I units fed distillate receiver effluent showed an 89% reduction in COD from an influent average of 326 mg/L to an effluent value of 35.5 mg/L. The increase in glyphosate concentration in the influent from <1 to 25 mg/L appeared to have no effect on COD removal.

During the period in which Condition II units ran at 24 hour retention time, the units showed a 75% reduction in COD from an influent average of 770 to an effluent value of 195 mg/L. When these units were run at a 48 hour retention time, performance deteriorated slightly. COD removal dropped to 56%. This deteriorating performance reflected in part a loss of solids from the units. During the 48 hour retention period, no sludge was intentionally wasted from the aerators. At these retention times the food to microorganism ratio drops to levels that favor increased endogenous respiration at the expense of sludge production. The settling characteristics of the sludge are also increasingly disrupted to the point that flocculation aids such as cationic polymers may be required in the sedimentation basins. Since air driven bench scale biological units have a relatively high energy input per unit volume, these units with their small settling sections are also more subject to settling disruptions than full scale systems.

As is noted in Figures 3 and 4, the COD of Conditions II and III influent showed an increase during the 48 hour retention test. The cause of this phenomenon is not clear. It is possible that the lime treatment was not carried out as efficiently during the later part of the study. Condition I influent, which did not receive centrifuge wash, showed no comparable increase.

During the period in which Condition III units ran at 24 hour retention time, COD reduction average 72%. Influent values averaged 1,450 mg/L and effluent values averaged 413 mg/L. COD reduction during the 48 hour retention time test was 64%. Influent and effluent COD averages were 1,550 and 557 mg/L, respectively.

Table 3 presents MLVSS data for the biological treatment units. Condition I units showed a steady drop throughout the study reflecting a

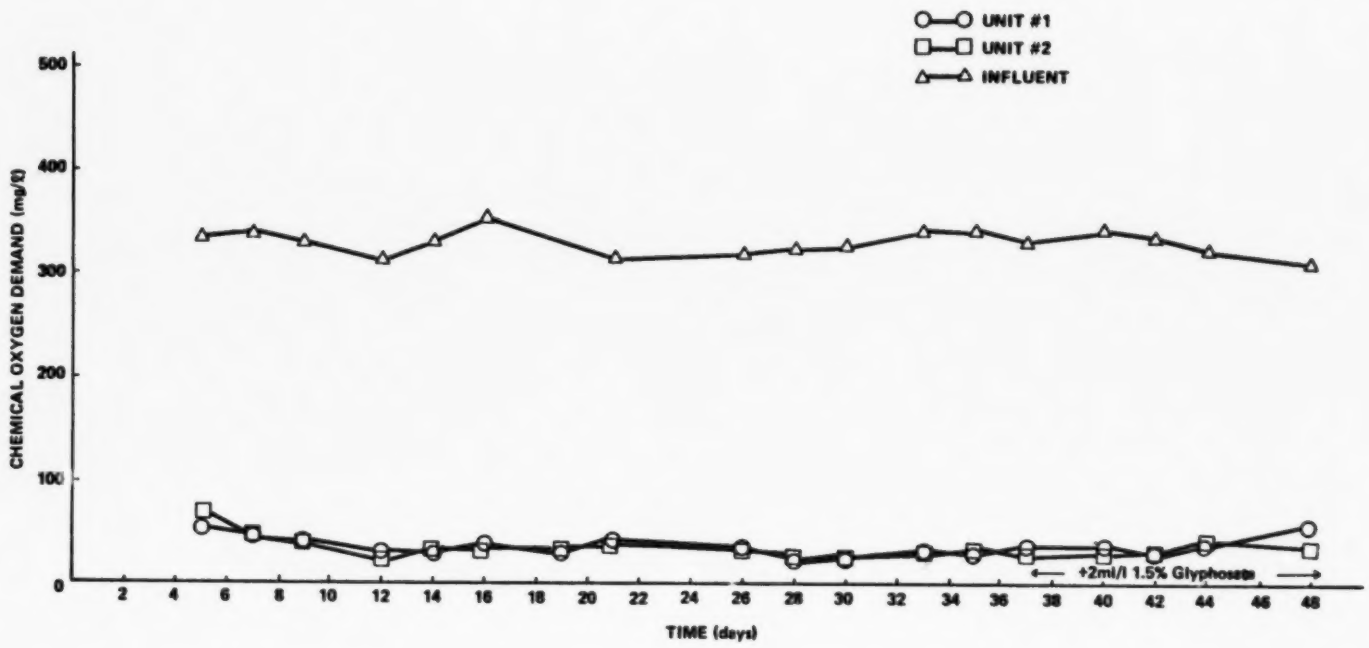


Figure 2. Influent and effluent COD levels for Condition I biological treatment units.

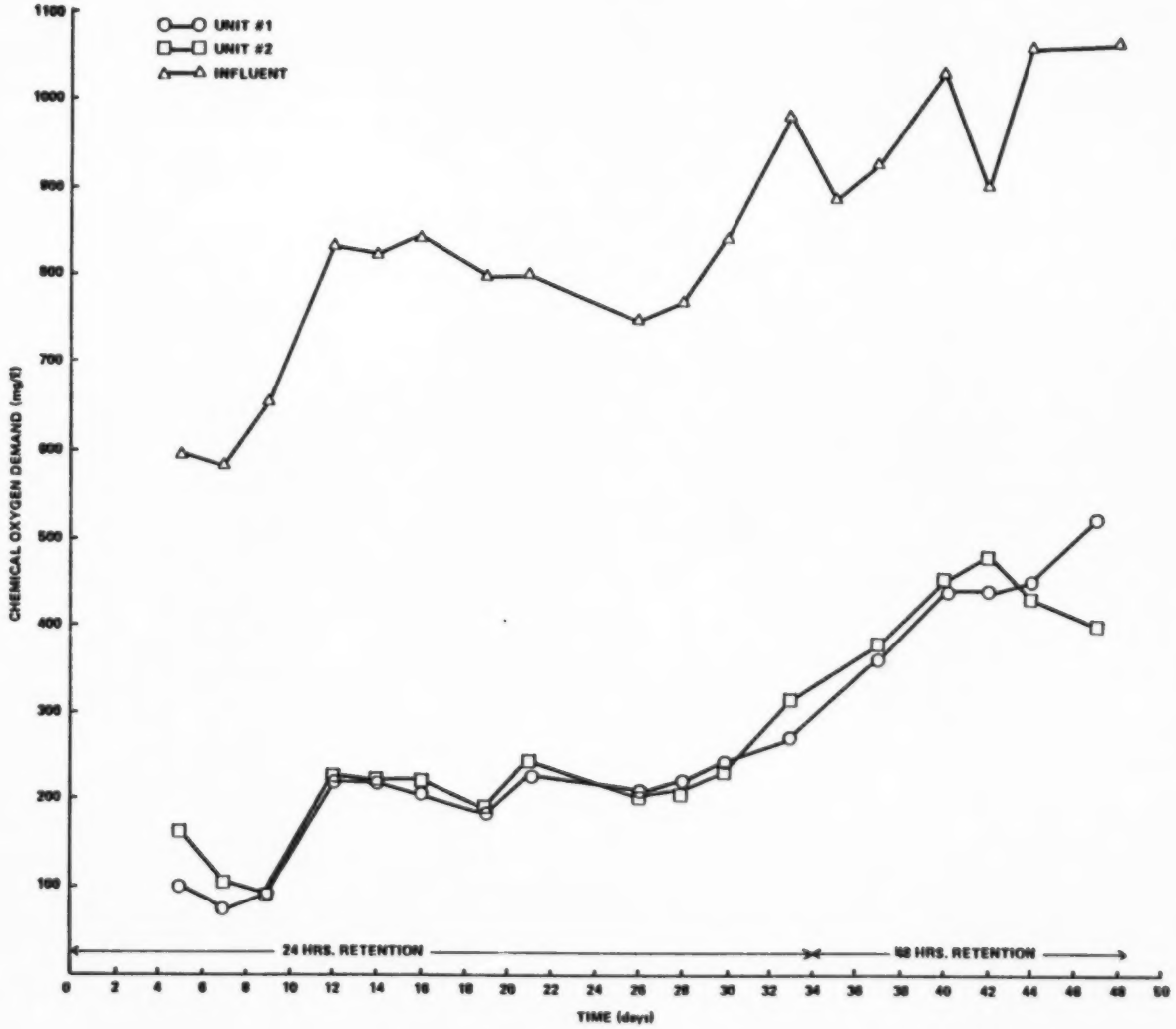


Figure 3. Influent and effluent COD levels for condition II biological treatment units.

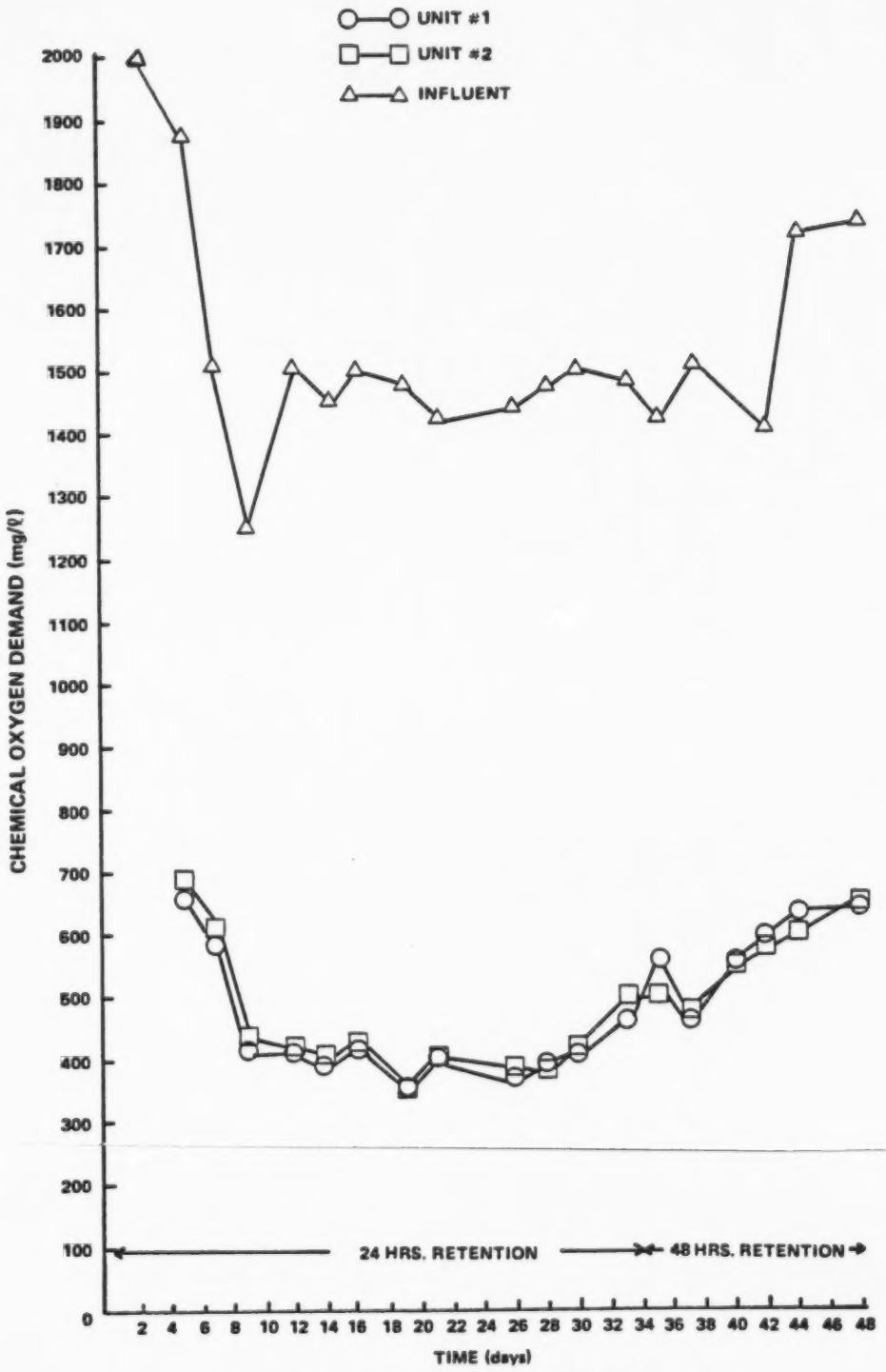


Figure 4. Influent and effluent COD levels for condition III biological treatment units.

TABLE 3. MLVSS DATA FOR BIOLOGICAL TREATMENT UNITS

	Condition I		Condition II		Condition III	
Day	Unit 1 (g)	Unit 2 (g)	Unit 1 (g)	Unit 2 (g)	Unit 1 (g)	Unit 2 (g)
1	4.09	3.68	4.36	3.99	4.39	4.40
6	3.36	3.22	3.90	3.54	4.86	4.92
13	2.72	2.82	4.65	3.82	5.55	5.37
20	2.66	2.77	5.02	4.28	7.18	6.99
27	2.14	2.14	3.80	3.68	6.33	6.18
34	1.87	2.12	3.38	3.13	4.76	4.65
37	1.99	1.92	2.81	2.75	4.63	4.94
41	1.71	1.68	2.37	2.65	4.29	4.57
48	1.61	1.70	2.35	2.55	3.75	4.10

pattern of endogenous respiration. However, the MLVSS-MLSS ratio improved to 0.5 percent. The food to microorganism ration (F/M) initially averaged 0.08 day^{-1} which is quite low. With the drop in solids the F/M ratio increased to 0.2 day^{-1}. A more efficient way to increase F/M ratio would be to decrease retention time of the feed solution. However, the longer retention times were designed to increase the degradation of glyphosate.

More control over solids levels could be achieved with Condition II and III units during the 24 hours retention time test. Sludge production rates were high particularly in Condition III. During the 48 hour retention time test run, solids production fell off markedly in Condition II units.

One notable comparison should be made between the solids levels in Condition II and III units which indicates a significant difference in response to lime-treated glyphosate wash. Influent to both sets of units contained a lime-treated glyphosate wash which it can be presumed was at least saturated with calcium salts. These salts apparently accumulated in the aerator portion of the biological treatment units. Eventually, a fine, sand-like, white precipitate collected in the sludge

return line of the Condition II units, where it could be removed. No such precipitate was noted in the Condition III units. Rather these solids appeared to stay in a type of colloidal suspension, imparting a lighter hue to the sludge.

Suspended solids in Condition III units increased from initial levels of 10,000 to 11,000 mg/L to concentrations of about 20,000 mg/L when corresponding MLVSS levels reached 7,000 mg/L. Increased wasting of solids brought the MLSS and MLVSS levels back down to about 15,000 and 4,000 mg/L, respectively. Despite the increased sludge wastage the MLVSS-MLSS ratio in the Condition III units continued at $\sim$ 0.37. The ratio for Condition II units on the other hand increased to 0.5 with the selective removal of calcium precipitates.

Increased sludge age which is typical of long feed retention times and low F/M will compound the problem of the accumulation of nonbiodegradable, inorganic solids. The danger of this accumulation is that inorganic solids levels can reach a level that interferes with biological activity and the treatment of the waste. In the present example, the high level of calcium salts might also provide a positive interference in the determination of MLVSS. In the final ignition step of this determination which occurs at 550°C (APHA, 1975), CO_2 can be lost during the following reaction:

$$CaCO_3 \rightarrow CaO + CO_2\uparrow$$

Under most circumstances the solubility of $CaCO_3$, is such that the interference would not be great. However, in the case of Condition III the calcium salt concentration in suspension may be on the order of grams per liter which could cause a significant overestimation of the amount of viable solids in the aerator.

Oxygen consumption levels in the three sets of units was fairly constant. Oxygen consumption corrected for solids levels and expressed as mg O_2 per gram solids per hour is shown in Table 4.

It can be concluded from the operational and performance data presented above that the biological treatment units were operating well given the wastewaters under treatment. It could then be concluded that glyphosate concentrations contained in the influents did not interfere

with the biological treatment process. On the other hand, glyphosate itself did not show a pattern of ready degradation. Glyphosate levels in the effluent ranged from 56 to 68 mg/L in Condition II during the 24 hour retention time test (100-105 mg/L in the influent). Condition III effluent glyphosate concentration ranged from 59 to 72 mg/L during the 24 hour retention time test. Condition I effluent values ranged from 10 to 23 mg/L. During the 48 hour retention time test with Condition II and III there was only minimal reduction in glyphosate concentration after treatment. Glyphosate values for Condition II and III effluents varied from 92 to 100 mg/L.

TABLE 4. OXYGEN CONSUMPTION IN BIOLOGICAL TREATMENT UNITS

Day	Condition I		Condition II		Condition III	
	mg/g/hr		mg/g/hr		mg/g/hr	
6	3.21	4.29	4.15	2.71	6.54	5.73
12	4.41	4.26	3.10	3.46	3.89	3.91
19	3.61	4.55	3.59	4.35	4.35	3.86
27	5.89	5.33	6.00	7.01	4.83	4.85
43	3.96	3.32	5.87	4.05	6.35	6.80

It is not clear whether the reduction in glyphosate concentration during biological treatment was a function of biological degradation or a physical-chemical sorptive process. In order to distinguish between these two mechanisms an oxygen uptake study was conducted with sludge spiked with two different concentrations of glyphosate. Sludge from a Condition II unit was aerated for 15 minutes to bring oxygen level to saturation. To 31 mL of sludge from unit 1 was added 1 mL of 125 mg/L glyphosate solution. To 31 mL of sludge from unit 2 was added 1 mL of 6,000 mg/L glyphosate solution. As a control for each test condition, 1 mL of deionized water was added to 31 mls of sludge from the corresponding

units. After addition of test or control solutions, the test vessel was sealed with an oxygen probe (Yellow Springs Instrument probe and meter) and stirred with magnetic stir bar at a constant rate. Oxygen readings were taken until the oxygen levels were reduced to 20% of initial levels. Little differences was seen in the oxygen consumption rate of either the test units or their respective controls. Oxygen consumption rate of the unit fed 125 mg/L glyphosate was 0.1944 mg/L/ min and its control was 0.1910 mg/l/min. The rate for the unit fed 6,000 mg/L glyphosate solution was 0.1498 mg/L/min and its control was 0.1530 mg/L/min. While the test does not provide any evidence for metabolic uptake of glyphosate, it is also interesting to note that fairly high concentrations of the compound do not inhibit other microbial processes in acclimated sludge.

If sorbtion is the major mechanism for removal, an explanation may be possible for the lack of reduction during the 48 hour retention time test. Because the sludge production was low with a corresponding low rate of wastage the solids may have become saturated with respect to glyphosate. As a result little sorption of glyphosate would occur and glyphosate would instead pass through the units.

TOXICOLOGICAL EVALUATION OF GLYPHOSATE WASTEWATERS BEFORE AND AFTER TREATMENT

Toxicological testing of the various influents and effluents was conducted with the algal species Selenastrum capricornutum and the invertebrate Daphnia magna. The methods of testing are detailed in Appendices D and E.

An overall pattern of diminished aquatic toxicity after biological treatment was exhibited by all three conditions. As shown in Table 5,

TABLE 5. EFFECT OF CONDITION I INFLUENT ON ALGAL GROWTH

Condition I Influent (mL/L)	0.01	0.1	1.0	10	100
Growth (% of control on day 14)	90	89	90	58	0.06

the algal EC_{50} (concentration effective in reducing growth to 50% of controls) of Condition I influent was approximately 10 mL/L. Algal assays on Condition I influents and effluents occurred prior to time when influents were spiked with glyphosate. Glyphosate concentrations were less than 1 mg/L. Condition II influent showed a toxicity pattern very similar to Condition I. As shown in Table 6, the EC_{50} of Condition II was also approximately 10 mL/L.

All effluents showed reduced toxicity compared to influents. As shown in Tables 7 and 8, the EC_{50} for both Condition I and II effluents was between 560 and 1,000 mL/L. The similarity in toxic response of algae to Condition I and II influents and effluents would seem to indicate that the presence of glyphosate in concentrations of 60 to 105 mg/L has little effect on algal growth. As will be discussed below, the toxic effects of glyphosate are somewhat dependent on water quality parameters such as calcium, magnesium, and carbonate concentrations.

Condition III influent was more toxic than either Condition I or II. As shown in Table 9, the EC_{50} for Condition III influent was between 1.0 and 10 mL/L. It should be noted that the intermediate wash component of Condition III was not lime treated as normally carried out during manufacture of glyphosate. Table 10 shows that EC_{50} for Condition III slightly more toxic that Condition I and II effluents. The toxicity of Condition III effluent was significantly reduced as compared to untreated influent. As noted above with Condition I and II, glyphosate does not appear to be the principal reason for the influent toxicity since its concentration is only marginally reduced in the effluent.

Toxicity testing with daphnia showed a pattern similar to the algal assays. As shown in Table 11 the LC_{50} (concentration which is lethal to 50% of the test population) of Condition I influent was between 32 and 56 mL/L. For Condition II the LC_{50} was between 18 and 32 mL/L as shown in Table 12. Biological treatment eliminated the short-term toxicity of Condition I and II effluents. Undiluted effluents from these two conditions showed no short term toxicity to Daphnia magna.

TABLE 6. EFFECT OF CONDITION II INFLUENT ON ALGAL GROWTH

Condition I Influent (mL/L)	0.1	1.0	10	100
Growth (% of control on day 14)	88	95	61	0.43

TABLE 7. EFFECT OF CONDITION I EFFLUENT ON ALGAL GROWTH

Condition I Effluent (mL/L)	56	100	180	320	560	1,000
Growth (% of control on day 14)	98	84	65	60	58	8.1

TABLE 8. EFFECT OF CONDITION II EFFLUENT ON ALGAL GROWTH

Condition II Effluent (mL/L)	56	100	180	320	560	1,000
Growth (% of control on day 14)	107	83	67	58	74	3

TABLE 9. EFFECT OF CONDITION III INFLUENT ON ALGAL GROWTH

Condition III Influent (mL/L)	0.1	1	10	100
Growth (% of control on day 14)	105	120	0.7	0.8

TABLE 10. EFFECT OF CONDITION III EFFLUENT ON ALGAL GROWTH

Condition III Effluent (mL/L)	100	180	320	560	1,000
Growth (% of control on day 14)	73	64	61	19	0.39

TABLE 11. EFFECT OF CONDITION I INFLUENT ON DAPHNIA MAGNA SURVIVAL

Condition I Influent mL/L	10	18	32	56	100	control
% Survival at 48 hours	90	100	80	0	0	90

TABLE 12. EFFECT OF CONDITION II INFLUENT ON DAPHNIA MAGNA SURVIVAL

Condition II Influent mL/L	10	18	32	56	100	control
% Survival at 48 hours	100	100	40	0	0	100

The LC_{50} of Condition III influent was between 32 and 56 mL/L as shown in Table 13. The LC_{50} of Condition III effluent was between 560 and 1,000 mL/L as shown in Table 14.

TABLE 13. EFFECT OF CONDITION III INFLUENT ON DAPHNIA MAGNA SURVIVAL

Condition III Influent mL/L	10	18	32	56	control
% Survival at 48 hours	100	100	100	0	100

TABLE 14. EFFECT OF CONDITION III EFFLUENT ON DAPHNIA MAGNA SURVIVAL

Condition III Effluent mL/L	180	320	560	1,000	control
% Survival at 48 hours	100	100	100	0	90

Because Condition III effluent most closely represents the total effluent from production of glyphosate, it was decided to run a daphnia reproduction bioassay with this effluent to further test the implication of the discharge of this type of wastewater on aquatic organisms. The details of this bioassay are presented fully in Appendix E.

Because of difficulties with the controls this bioassay did not meet the criteria for absolute validity. However, the results highly suggest that the wastewater is relatively low in toxicity. Daphnia raised in 30% concentrations of wastewater showed a reproduction rate higher than the rate for the typical brood stock raised in our laboratory under optimal conditions. Daphnia raised at 50% concentrations showed a reproduction rate approximately 34% that of the daphnia raised in 30% concentrations. Daphnia raised in 75% concentrations of Condition III effluents showed good survival over a two week period but almost no reproduction.

As noted in Appendix E, all daphnia assays are conducted in water with a hardness of 160 to 180 mg/L as $CaCO_3$. Because of the ionic character of the glyphosate molecule, it was hypothesized that toxicity would vary with water hardness. To test this hypothesis separate bioassays were conducted with reference grade glyphosate (99+% purity) in both hard and soft water. Appendix E contains the formula for reconstituting deionized water as either hard or soft water.

As can be seen in Table 15 the LC_{50} of glyphosate in soft water was between 32 and 56 mg/L and LC_{50} in hard water was greater than 100 mg/L. The results for soft water correspond closely to results obtained by Folmar et al., (1979) for the effect of technical grade glyphosate on the invertebrate Chironomous plumosus.

TABLE 15. COMPARISON OF TOXICITY OF GLYPHOSATE TO DAPHNIA MAGNA IN HARD AND SOFT WATERS

Glyphosate concentration mg/L	100	56	32	18	10	0
% Survival in hard water at 48 hours	100	100	100	100	100	100
% Survival in soft water at 48 hours	0	0	100	100	100	100

Algal assays were also conducted to test the effect of glyphosate on Selenastrum capricornutum. As shown in Table 15 the EC_{50} of glyphosate in pure solutions is approximately 10 mg/L.

At least two theories have been developed to account for the decrease in toxicity of many ionic compounds with increasing water hardness. In one theory calcium complexes with the toxicant rendering it less available to the algal cell. According to a second theory carbonate may compete with the toxicant for available sites on the cell surface. In either case, it is interesting to note than in the successive dilutions of the test solutions for the algal assay, as the toxicant is being diluted, so also are the competing elements being diluted. This may account for the phenomenon depicted in Tables 7, 8, and 10. As test solution is diluted, growth seems to plateau at a rate less than control. It may be that in this middle range of dilution values the actual toxicant concentration the organism "sees" is constant.

Several conclusions relevant to the discharge of glyphosate production wastewater can be drawn from the toxicological testing presented above. Glyphosate in soft water appears to be more toxic than glyphosate in a biologically treated wastestream. This decreased toxicity in wastewaters is likely due to the addition of the lime-treated glyphosate wash to the total effluent mixture with a resulting increase in calcium carbonate in the effluent. Toxicity of glyphosate in production wastewater could vary with the characteristics of the receiving water. The toxicity of glyphosate could be expected to be less in harder receiving waters.

SUMMARY OF BIOLOGICAL TREATMENT RESULTS

A review of the three treatment conditions indicates that the glyphosate concentration exists rather independently of biological treatment. Distillation receiver wastes, as represented by Condition I, were easily biotreated with regard to most organics present with the exception of glyphosate. The effect of increased concentration should be investigated. Higher concentrations of glyphosate in Condition II were not significantly degraded but did not interfere with biodegradation of other organics. Condition III represents a mixture which is qualitively quite similar to a total production wastestream. While the glyphosate

intermediate concentration was similar to that in the influent of Monsanto's system this concentration was obtained by dilution rather than lime treatment. The Condition III mixture, with the addition of intermediate, was in large part readily biotreatable, though the glyphosate concentration was only partially reduced as in Condition II. Biological treatment of all three conditions substantially reduced toxicity of effluents relative to influents.

It would, however, be difficult to define a typical daily or even weekly flow from the production of glyphosate. The real source of variability in any treatment scenario is the input glyphosate and its intermediate from "spills, leaks, and washdown". A study of wastestream discharge records indicates that these contributions could be substantial. It may well be that the most effective form of treatment would be to minimize this input.

ADDITIONAL TREATMENT OPTIONS

Several other treatment options were investigated in the search for methods to reduce glyphosate concentration. These options included: ozonation, adsorption (resin and carbon), and ion exchange.

Ozonation

Theoretically ozone could be used as the sole method of treatment for many industrial wastewaters. However, the cost of complete ozone oxidation of waste organics in most mixed wastestreams makes this treatment infeasible.

Ozone is more economically applied in combination with other treatment methods. In its well-studied role as drinking water oxidant-disinfectant ozonation is used as a final polishing step after the major removal of organics and pathogens has occurred through coagulation, flocculation, and sedimentation.

Ozonation has been used in conjunction with activated carbon treatment. Preliminary ozonation is said to improve activated carbon performance. The mechanism of improvement includes possible increased adsorbitivity of oxidized organics and increased biological degradation of adsorbed organics at the carbon surface (Guirguis et al., 1978; Randtke, 1978).

Ozonation has also been used to increase the biodegradability of complex industrial wastewaters. Several highly toxic and refractory textile dye waste effluents showed increased biodegradability after ozonation (Netzer and Miyamota, 1975). Ozone pretreatment may be something of a two-edged sword since it was found that several more biodegradable textile waste effluents became less treatable after ozonation. This may be explained by the fact that the more degradable compounds were oxidized preferentially by the ozone (Netzer and Miyamota, 1975).

Oxidation of pesticides in pure solutions by ozone with and without ultraviolet (UV) irradiation has been studied (Prengle and Mauk, 1978). Ozone used in conjuction with UV increased the destruction of the pesticide when compared to ozonation alone. Unfortunately it is difficult to make predictions on the oxidation of pesticides in complex mixtures from data on pesticides in pure solutions.

Two studies of the ozonation of glyphosate were conducted. The first study investigated the oxidation of glyphosate in a pure solution. One liter of technical grade glyphosate solution with a measured glyphosate concentration of 44.6 mg/L was placed in a one liter graduated cylinder. Ozone, supplied by a Wellsbach T-23 ozone generator, was pumped through Teflon tubing and a stainless steel diffuser placed at the bottom of the cylinder. Ozone production rate was 42 mg/minute as standardized with a KI solution. Twenty mL aliquots were withdrawn every 30 minutes and analyzed for glyphosate.

In the apparent presence of a constant or excess concentration of ozone destruction of glyphosate followed first order kinetics with respect to glyphosate concentration. Figure 5 presents a plot of log concentration versus time for the destruction of glyphosate. Table 16 presents the data from which this plot was taken.

TABLE 16. OZONATION OF GLYPHOSATE

Time (hour)	Glyphosate mg/L
0	44.6
0.5	27.0
1.0	15.3
1.5	6.5
2.0	3.5
3.0	0.0

The time constant K for the reaction, as computed from the slope of -0.0095, was 0.0219 min^{-1}. The half-life of glyphosate under these conditions was about 32 minutes. Such a long half life does not give a good prognosis for economical ozonation of this compound.

To test the oxidation of glyphosate in a complex matrix one liter of Condition III effluent was ozonated under the same conditions presented in the previous experiment. The original concentration of glyphosate was 92 mg/L. After 2 hours of ozonation glyphosate concentration was reduced to 80 mg/L. It can be concluded that ozonation does not provide a feasible alternative for the reduction of glyphosate. Whether ozonation might increase the biodegradability of the residual organics in the biologically treated effluents from glyphosate manufacture could not be tested within the time constraints of this project.

Adsorption

Due to the ionic character of the glyphosate molecule, carbon adsorption does not provide a feasible alternative for the removal of glyphosate from waste streams. On the contrary it is reported that powdered activated carbon is used during sample "clean up" prior to analysis for glyphosate without apparent effect on glyphosate recovery (M. Rueppel, 1980).

To test for adsorption of glyphosate on various synthetic adsorbents, batch tests were run with the absorbents XAD-4, XE-340, and XE 348 as manufactured by Rohm and Haas. Of the absorbents used here, XE 348 is most similar to activated carbon (Rohm and Haas, 1977).

Each resin was soaked in methanol for 30 minutes and then rinsed well with several volumes of water. To 100 mL aliquots of filtered (Whatman 2V) Condition III effluent were added either 2 mL or 0.2 mL of each of the absorbents. In addition, a blank was carried through all steps of the procedure. All samples were shaken at 110 oscillations per minute for 90 minutes. At the end of this period the resin was allowed to settle and the supernatant decanted and analyzed for glyphosate. Table 17 presents results of this study. Results of these batch tests indicate relatively little adsorption of glyphosate on these synthetic adsorbents. It should also be noted that batch tests typically overestimate the capacity of large scale column systems.

TIME (min)	log C
0	1.65
30	1.43
60	1.18
90	0.81
120	0.54

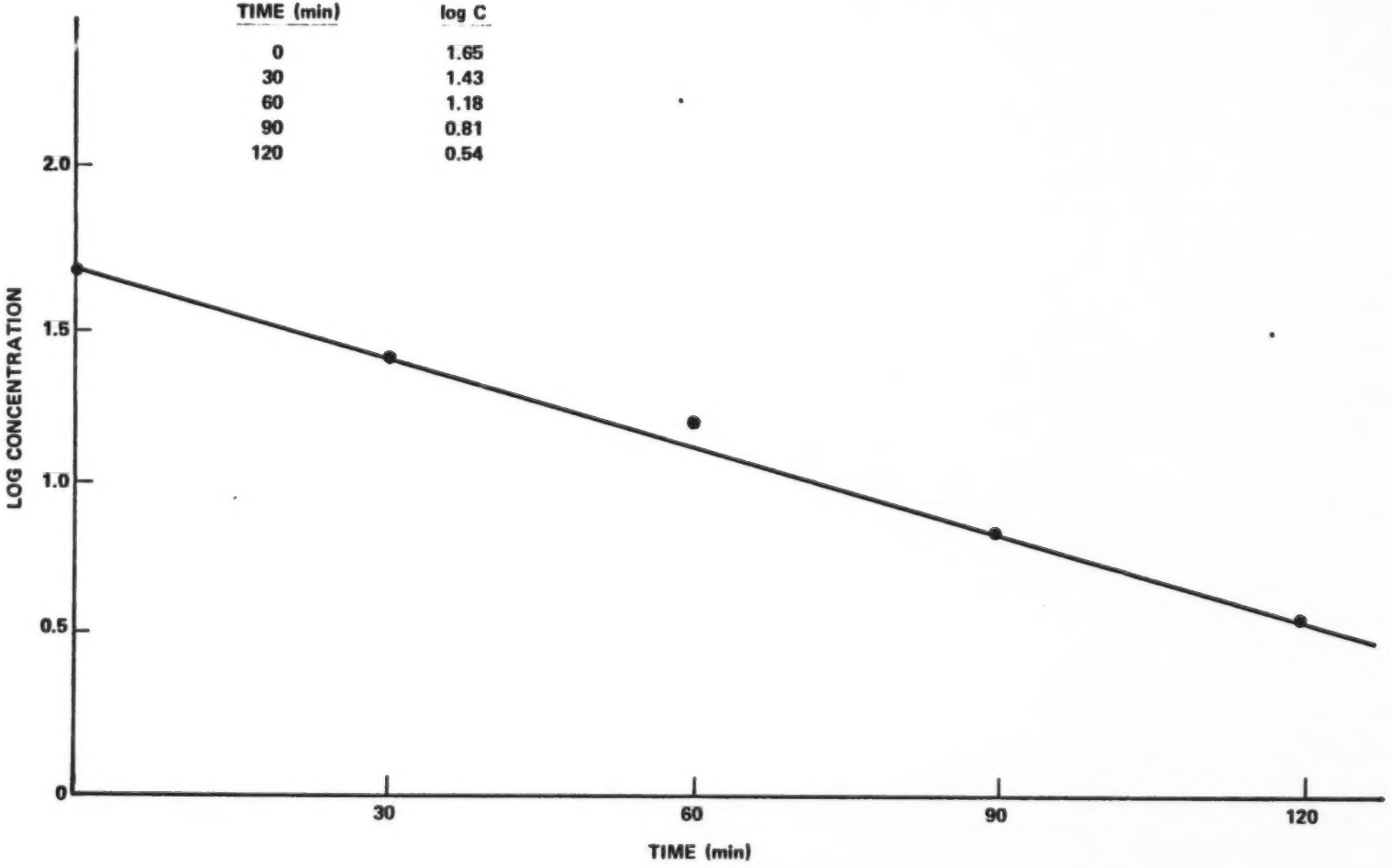


Figure 5. Rate of ozonation of glyphosate.

TABLE 17. ADSORPTION STUDY WITH CONDITION III EFFLUENT

Adsorbent	Glyphosate (mg/L)
XAD-4	
2.0 mL	38.0
0.20 mL	40.2
XE-340	
2.0 mL	37.6
0.20 mL	39.0
XE-348	
2.0 mL	36.9
0.20 mL	37.4
Blank	45.3

Ion Exchange

Considerable work has been done at RTI on the use of ion exchange resins to concentrate various compounds from aqueous solutions for subsequent analysis. It has been found that the concentration efficiency of ion exchange resins is highly dependent on the ionic strength of the solution from which the compound of interest is to be isolated. A compound such as glyphosate will be competing for available sites to some degree with the other ions in solution. In general, the exchange capacity of an ion exchange resin and the ionic strength of the water sample determine the volume of water which can be treated effectively. By taking conductivity measurements of various samples a prediction can be made of the volume of effluent which can be passed through an ion exchange column before breakthrough. Table 18 presents empirically determined relationships between conductivity and breakthrough volume for various types of effluents passed through an open tubular chromatography column filled with 10 mL of Biorad AG 1-X8 resin (Pellizzari, 1979). 60 mls of 0.67N $NaHSO_4$ in acetonitrile:water (2:1) were required to regenerate a column of this size. The elution volume of 0.67 N $NaHSO_4$ in pure water would be larger. In an ion exchange the treatment scenario this elution volume would depend upon the mineral acid and its

concentration. In practice H_2SO_4 at concentrations of 1-2 M might be used.

The conductivity of Conditions I, II, and III effluents was measured on an Industrial Instruments Conductivity bridge, model RC16BZ. The conductivity readings in μmhos/cm of the biologically treated effluent from the three conditions were: I, 940; II, 1,620; III, 5,640. Based on the results presented in Table 18 ion exchange treatment of Conditions III and II is infeasible. With regeneration of the resin with a strong mineral acid and no degradation of the glyphosate, the waste disposal problem would be worse unless large concentration factors can be realized. Since it is clear that Conditions II and III will experience small, if any, concentration factors, ion exchange has little applicability to these waters.

TABLE 18. ESTIMATED IONIC EQUIVALENTS AND BREAKTHROUGH VOLUMES OF TEST WATERS

Water type	Conductivity (μmho/cm)	Volume before breakthrough (mL)[a]
distilled-deionized	0.5	-
drinking water	136	2,500
municipal effluents	485	500
energy effluents		
1	1,530	166
2	25	8,333
3	2,720	100
4	11,000	25
5	2,100	125
6	2,700	100
7	3,470	83
8	1,570	166
9	3,830	70
10	2,210	125

[a]Ion exchange columon filled with 10 mL of Biorad AG 1-X8 resin (1.4 meq/mL).

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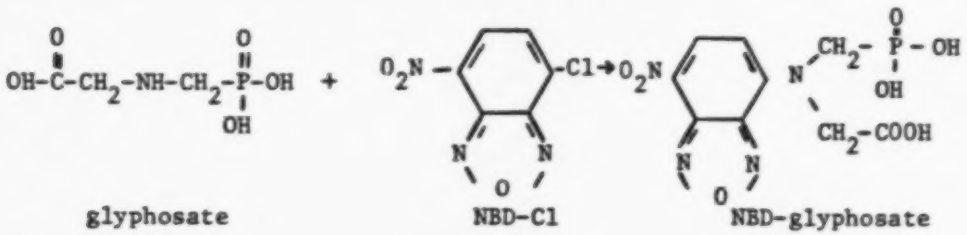
APPENDIX A

ANALYTICAL METHOD FOR DETERMINATION OF GLYPHOSATE

The procedure for the analysis of glyphosate involves derivatization of glyphosate with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), followed by separation and quantitation of the derivative by high-performance liquid chromatography (HPLC).

The derivatization procedure is a modification of the method of Lawrence and Frei (1972). NBD-Cl reacts with alkyl amines in aqueous solution to form the NBD-amine derivative:



glyphosate NBD-Cl NBD-glyphosate

0.2 ml of 1% NBD-Cl in MeOH 2nd 0.2 ml 0.1 N $NaHCO_3$ were added to 0.2 ml wastewater in a small test tube. The tube was loosely stoppered, shaken, and heated in a water bath at 80°C for 30 min. The solution was cooled, filtered through a Millipore LS filter, and a 10 µl aliquot removed for analysis.

A modular liquid chromatographic system consisting of the following components was used for analyses: 2 M6000 pumps with a M660 solvent programmer and a U6K injector (Waters Associates) and a Model SF-770 variable wavelength ultraviolet detector (Schoeffel Instruments).

Paired-ion chromatography, utilizing a reverse phase column packed with LiChrosorb RP-18 packing (Merck) and a CO:PELL ODS pre-column (Whatman) was used to separate and quantify the NBD derivative of glyphosate. A 40% MeOH/H_2O mobile phase with 0.005 M tetrabutylammonium hydrogen sulfate added as a counter-ion was used. The pH of the mobile phase was adjusted to 7.0 with 10% NH_4OH. Solvent flow was 2.0 ml/min and the variable wavelength detector was set at 475 nm. Minimum detectable quantity was 50 ng with a standard deviation of 1.2% at 5 mg/liter.

The chromatograms of Conditions II and III influents showed a large peak preceding the glyphosphate derivative peak. Complete resolution of the two peaks was not possible. Concentration of glyphosate were verified by the method of standard additions. Accurate values were determined by plotting peak areas of samples spiked with glyphosate to give concentrations 2 and 33 times the estimated glyphosate concentration. Values for unspiked samples could then be verified by extrapolation.

APPENDIX B

ANALYTICAL PROCEDURES FOR ROUTINE WASTEWATER CHARACTERIZATION

Routine wastewater analyses were conducted according to Standard Methods for the Examination of Water and Wastewater, 14th Edition, (APHA, AWWA, WPCF, 1975).

pH--

pH was determined electrometrically by Method 424.

Chloride--

Chloride was measured by the mercuric nitrate method (Method 408 B).

Acidity--

Acidity, as $CaCO_3$, was determined by Method 402.

Alkalinity--

Alkalinity, as $CaCO_3$, was determined by Method 403.

Nitrogen Forms--

Total Kjeldahl nitrogen was determined after digestion, according to Method 42. Ammonia (NH_3-N) was determined by an acidimetric method as described in Sections 418 A and 418 D. Nitrite and nitrate nitrogen (NO_2-N, NO_3-N) were determined by the Devarda's alloy method (419 F).

COD--

Chemical oxygen demand (COD) was determined by Method 508.

Residues--

Suspended solids (SS) were determined by Method 208 D. Total solids (TS) were determined by Method 208 A. Total dissolved solids (TDS) were determined by Method 208 A. Settleable solids were determined by Method 208 F.

APPENDIX C

PROCEDURES FOR CONDUCTING ACTIVATED SLUDGE TREATABILITY TESTS

For biological treatability studies the bench scale, complete-mix, continuous-feed, activated sludge unit designed by Swisher (1970) was employed (Figure C-1). This unit has an aerator capacity of 0.4 L and a settler capacity of 0.075 L. The unit is made entirely of glass, avoiding the possibility of contamination by organics leaching from the container. Continuous feed to the units was supplied through Teflon tubing by a peristaltic pump to give the nominal retention time of 8 hours.

Routine determinations were made of dissolved oxygen, pH, mixed liquor volatile suspended solids in the aerator and COD, and pesticides in the influent and effluent. Dissolved oxygen was determined with an oxygen probe (Yellow Springs Instrument Co.).

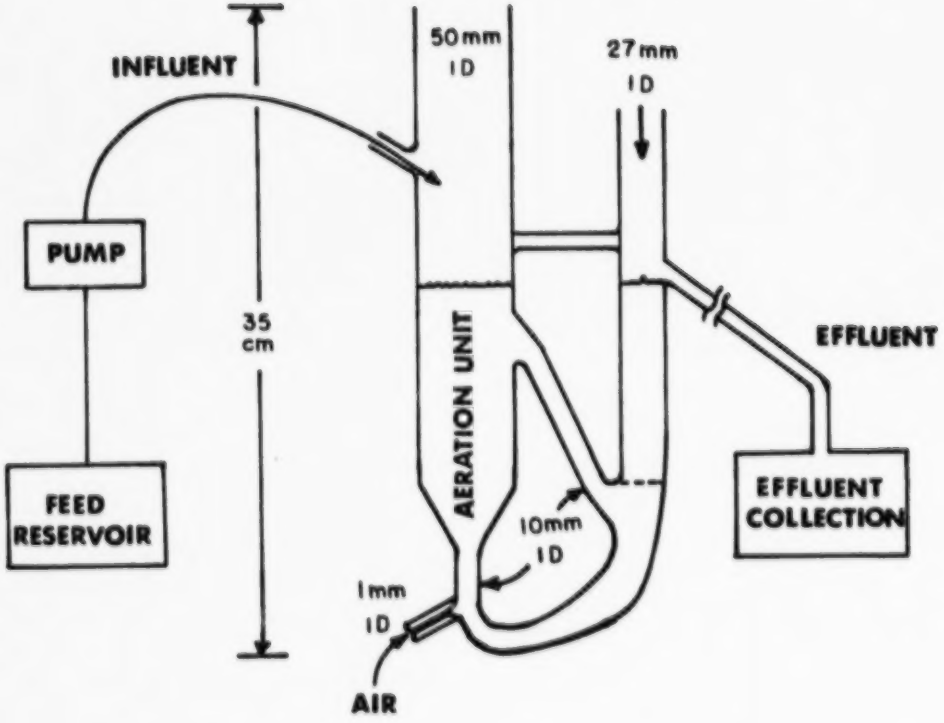


Figure C-1. Diagram of activated sludge pilot unit.

APPENDIX D

PROCEDURES FOR ALGAL ASSAY TESTS

Algal bioassays were conducted according to the freshwater algal assay procedure described in the report The Selenastrum Capricornutum Printz Algal Assay Bottle Test (Miller, Greene, and Shiroyama, 1978). The test alga was Selenastrum capricornutum Printz, obtained from the National Eutrophication Research Program, EPA, Corvallis, Oregon. This test was designed to measure algal response to changes in nutrient concentrations and to determine toxicity or inhibition.

Wastewaters to be tested were filter sterilized through a sterile prewashed membrane filter (Millipore Filter, 0.45 μm pore size). Serial dilutions were then made in sterile algal media to give the appropriate final concentration. Sufficient inoculum was added to produce an initial cell concentration of 10^4 cells/mL.

In each set of experiments, algal growth in the presence of a series of concentrations of the wastewater added to the nutrient medium was compared to algal growth in the nutrient medium alone. Growth was determined by direct counts of the algae during the 10-14 day incubation period. Effect of the wastewater on algal growth was determined in terms of the effect on the cell yield. Direct cell counts were performed by an automated procedure utilizing a Fisher Scientific Model FO 16 particle counter.

The tests were conducted in water bath shakers at 24 $\pm$ 2°C and at approximately 80 oscillations per minute with constant white fluorescent lighting at 4300 lux. Test containers were 250 mL Erlenmeyer Pyrex flasks containing 100 mL of test medium and covered with an inverted Pyrex beaker.

The method of expressing inhibitory or stimulatory effects was that recommended by Miller et al. (1978), i.e., as the percent growth inhibition (I) or stimulation (S), as compared to growth in a control culture without

the test materials. These authors suggest that, in general practice, the results be based on the growth at 14 days, i.e., as % I_{14} or % S_{14} at a given concentration of the effluent being tested. Typical cell counts for controls on day 14 of a test were between 5 and 6 million cells per mL.

Decreased growth, compared to the control, is evidence of an inhibitory effect. The manner in which the test is conducted does not allow determination of whether this inhibition is temporary (algistatic) or permanent (algicidal). Such a determination would require further testing by subculturing into fresh medium free of the test material.

APPENDIX E

PROCEDURE FOR DAPHNIA BIOASSAY TESTS

The 48 hour, static daphnia bioassay was adopted from the procedures outlined by Duke et al (1977). Daphnia magna was selected as the test species. An original population of Daphnia magna were obtained from W. T. Waller of the University of Texas at Dallas. The daphnia used in these bioassays are reared at Research Triangle Institute under conditions which promote parthenogenetic reduction. Each member of the breeding stock is reared in isolation. When reproduction occurs young are removed within 24 hours. Age of the breeding stock is staggered and each daphnia is replaced every 56 days. All daphnia in the present breeding population are the offspring of a single daphnid.

Daphnia are reared in deionized water which is reconstituted to standard hard water specifications by addition of 192 mg $NaHCO_3$, 120 mg each of $CaSO_4 \cdot 2H_2O$ and $MgSO_4$ and 8 mg of KCl per liter of water. Daphnia are fed daily portions of non-steril Selenastrum capricorntum except during testing periods.

Range finding bioassays may be conducted with a mixed population of daphnia. All narrow range, definitive bioassays are conducted with first instar young.

Tests are conducted in 250 ml beaker to which 100 mls of combined test solution and reconstituted hard or soft water has been added as noted in the test. When the bioassay required soft water, deionized water was reconstituted with the addition of the compounds given above for hard water at 25% of the concentration given above for hard water. Ten daphnia are randomly assigned to each condition. Survival of the daphnia at 24 and 48 hours is judged by careful examination for mobility. Survival of daphnia in the control condition must be 90% or better for bioassay to be considered valid.

A static bioassay testing the reproduction of daphnia exposed to Condition III effluent was conducted. Five daphnia were tested at each concentration. Three concentrations of Condition III biological treated effluent were tested. These conditions included 30%, 50%, and 75% effluent diluted in hard water. In each of 5 beakers per concentration containing 100 mL of the appropriate mixture was placed one first instar daphnia. Five daphnia placed in hard water alone were run as controls. Daphnia were fed 1 mL of Selenastrum Capricornutum every other day. Feed concentration was 10^7 cells per mL.

Because of difficulties in raising the controls this bioassay did not meet all provisions for validity. During the early days of the assay the controls only exhibited a phenomenon we referred to as "floating". The daphnid becomes trapped at the surface of the water and is held there by the surface tension of the water. This phenomenon has never affected the young of the stock breeding population. This "floating" phenomenon is also quite intermittent and occurs only at widely scattered intervals in our acute testing program. When it does occur it seems only to affect the control daphnia and the daphnia in the lowest concentrations of test solution while higher non-lethal concentrations are unaffected. When it does occur, most or all daphnia in these low contaminant conditions are affected. The problem is not unique to our laboratory. W. T. Waller of the University of Texas at Dallas reports using screens to keep daphnia below the surface during long term assays to avoid this phenomenon.

Where this phenomenon occurs during acute testing the bioassay is repeated. During the reproduction assay it was decided to let this phenomenon, which affected only the controls, run its course. After several molts the affected daphnia "recovered" but reproduction was greatly reduced. In the nineteen days of the assay the five controls produced only six broods of a total of 45 young. Daphnia tested in 30% Condition III biologically treated effluent produced 18 broods with a total of 322 young. Daphnia tested in 50% concentrations produced 110 live young and 7 dead young in 10 broods. Daphnia tested in 75% effluent showed 80% survival through 14 days and the production of only 2 broods of 4 live and 3 dead young. By comparison to the reproductive rates of

the controls used in this assay, the reproductive rate of 5 first instar young reared in our breed stock during the same 19 days showed a reproduction rate of 210 young in 12 broods.

Despite the difficulties with the controls it is obvious that the daphnia in 30% conditions thrived. This is probably a function of the increased nutrients in the form of microbial populations contained in a biologically treated effluent. Because of difficulties with controls the interpretation of the results of the 50 and 75% conditions more difficult. Though the daphnia in these conditions did not experience the same problems as controls, it might be argued that a general weakness pervading the daphnia population selected for this assay affected the resistance of these daphnia to toxicants.

APPENDIX F

DATA SUMMARY FOR GLYPHOSATE WASTEWATER TREATMENT CONDITIONS

Condition I: 1:20 distillate receiver waste in deionized water. Spiked with glyphosate to 25 mg/L from day 35 of biological treatment. COD of the influent averaged 326 mg/L. Glyphosate averaged < 1 mg/L to day 35; 25 mg/L after day 35.

Condition II: 37.5 mL of lime pretreated glyphosate wash brought up to 1 liter with Condition I influent. COD of influent averaged 770 mg/L during 24 hour retention time and 977 mg/L during 48 hour retention time. Glyphosate influent values were 100 to 105 mg/L.

Condition III: 9 mls of concentrated non-pretreated intermediate wash were brought up to one liter with Condition II influent. COD influent average 1450 mg/L during 48 hour retention time test.

As mentioned in the text the purpose of running three conditions was to test the degradability of glyphosate at increasing concentrations in increasingly complex waste mixtures. Based on discussions with Monsanto personnel and examination of effluent data from the manufacture of glyphosate the following scheme was worked out. Condition I would test the treatability of glyphosate at low concentration and in a relatively simple mixture. Because the information originally available indicated that the distillate receiver wastewater contained about 200 mg/L it was decided to dilute the stream 1 part in 19 parts deionized water to give a glyphosate concentration of 10 mg/L. If glyphosate proved to be biologically degradable at these concentrations could then be increased. Further analyses indicated lower than expected glyphosate concentrations and it became necessary to spike Condition I influent to a glyphosate concentration of 25 mg/L.

Conditions II and III were meant to test the degradibility of glyphosate in more complex mixtures with and without the addition of glyphosate-intermediate. It was decided to hold glyphosate concentrations initially to 100 mg/L which was determined to be the minimum possible glyphosate concentration which could be attained from a glyphosate production system. The glyphosate wash stream was added at a rate of about 3.7% to Condition I influent. If the glyphosate and associated waste organics were degraded, the glyphosate concentration in Condnition II influent could be increased by increasing the concentration of distillate receiver waste in the Condition I and II wastewater.

Condition III factors in the influence of glyphosate intermediate on the degradation of glyphosate and associated organics. While the intermediate wash is a relatively small flow stream, it is very concentrated stream before lime pretreatment. Intermediate wash prior to lime pretreatment was added at a rate of 9 mL per liter of Condition II influent. The concentration of the glyphosate and the glyphosate intermediate in Condition III is fairly representative of what might be expected in a glyphosate production waste stream.